REMARKS/ARGUMENTS

Status of the claims

Claims 79 to 84 and 91 to 99 were previously pending and presented for examination. Claim 79 was amended to delete the extraneous period objected to by the Examiner. Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry. After entry of these amendments, claims 79 to 84 and 91 to 99 will be pending.

Claim 79 stands objected to because of the following recital: "moiety is excited.;" wherein an extraneous period appears. As noted above, the Applicants have amended claim 79 to remove the offending period and accordingly request withdrawal of the rejection.

Claims 79-84 and 91-99 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13 and 43-44 of U.S. Patent No. 6,803,188. As noted in their previous Amendment, without acquiescing on the merits, in the event that claims are allowed in this application and a terminal disclaimer must be provided, Applicants would submit a Terminal Disclaimer in accordance with 37 C.F.R. 1.132(c). Applicants therefore request that the rejections of be held in abeyance until such time as a claim is otherwise deemed to be in condition for an allowance.

Claims 79-81, 91-94, 97 and 99 remain rejected under 35 U.S.C. 112, first paragraph, for an alleged lack of enablement. Claims 14, 15, 17 to 23, and 25 to 29 stand rejected under U.S.C. §112, first paragraph as allegedly failing to comply with the enablement requirement. Applicants next respectfully traverse the rejection.

To further address specific concerns set forth in the Action, the Applicants supplement their earlier *Wands* analysis by providing additional remarks concerning the specific concerns with respect to enablement. 1) the length of the polypeptide linker, 2) the posited fluorescent linker; 3) the protease recognition site subject matter; 4) the cyclization and oxidation subject matter; and 5) the scope of the allowed substitutions. We next address each of these concerns, tackling the simpler concerns first.

We start with the length of the polypeptide linker.

Length of the polypeptide linker

The Applicants thank the Examiner for again acknowledging that linkers from 5 to 50 amino acids in length are enabled. The Office Action indicated that the claims embraced longer linkers as evidenced by the open "comprising" language of claim 87. However, claim 87 had already been canceled. We also note that the actually pending claims were "closed" with regard to the polypeptide length of the linker. As previously amended, the base claims set forth that the linker, including the protease cleavable portion thereof, was from 5 to 50 amino acids in length. Accordingly, the Applicants believe this concern does not apply to the pending claims.

The posited fluorescent linker

We next turn to the concerns regarding any linker polypeptide which might be fluorescent. In contending that the claims embrace fluorescent linkers, the Examiner originally looked to the first full paragraph of p. 8 as indicating that the linker would be fluorescent. We believe this contention to be wholly inconsistent with the wording of that paragraph:

> "Moiety" refers to the radical of a molecule that is attached to another moiety. Thus, a "fluorescent protein moiety" is the radical of a fluorescent protein coupled to the linker moiety. By the same token, the term "linker moiety" refers to the radical of a molecular linker that is coupled to both the donor and acceptor protein moieties.

The first line of that recital, clearly indicates that the moiety which is the radical of a molecule does not embrace the *another* moiety but is attached to it. The recital of the fluorescent moiety of the second line of the recital begins with the recital "thus" indicating what follows is an application of the guidance provided by the preceding sentence. The second sentence accordingly is properly construed to indicate that the fluorescent protein moiety is coupled to *another* moiety, the linker moiety. As such, the fluorescent moiety of the second sentence does not include the linker moiety but is attached to it. The third sentence of the recital, begins with "by the same token" to indicate what follows is an application again of the same guidance provided in the first sentence of the paragraph. Accordingly, this last sentence clearly sets forth the linker moiety, the donor fluorescent moiety, and the acceptor fluorescent moiety as three

distinct moieties. Nothing in this passage can be fairly construed as teaching that the linker moiety is itself fluorescent. Indeed, of the many linker moieties set forth in the specification, *none* are indicated to be fluorescent.

Assuming *arguendo* that it may be postulated that the above linker recital does not necessarily exclude polypeptide linkers which are fluorescent and capable of frustrating the fluorescent resonance energy transfer between the recited acceptor and donor fluorescent moieties of the claims, we note that the base claims expressly exclude any such postulated inoperative embodiments in view of their functional recitals "wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited."

Moreover, the Office Action posits fluorescent linker subject matter that one of ordinary skill in the art would recognize is opposed to the functioning of the invention as expressly taught throughout the specification. With respect to the alleged existence of these inoperative embodiments falling within the scope of the claims, the standard for enablement in the presence of inoperative embodiments is not whether one can imagine an inoperative embodiment. Rather, pursuant to MPEP § 2164.08(b), the standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984) (prophetic examples do not make the disclosure nonenabling). Here, for reasons taught in the specification and relied upon by the Examiner in framing the rejection, the skilled person would certainly know to avoid the fluorescent linker embodiments postulated by the Examiner.

Thus, noting that 1) the above-quoted passage does not teach the postulated inoperative fluorescent linkers; 2) the claims exclude the presence of the postulated inoperative fluorescent linkers by setting forth the functional requirement that "the donor fluorescent protein moiety and the acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited;" and 3) persons of ordinary skill

in the art would know to avoid such inoperative fluorescent linkers in view of the teachings of the specification, the Applicants submit that the linker subject matter of the claims can be readily practiced by persons of ordinary skill in the art.

The protease recognition site subject matter

The Office Action appears to contend that the subject matter of the protease recognition sites lacks enablement. In this regard, we note that the specification teaches 14 proteases as being suitable and assert that the state art of the pertinent protease cleavable linker art is high. As evidence thereof, we show that the Patent Office has granted many claims which generically set forth protease cleavable linkers. See, the attached search of the PTO patent database for the occurrence of the terms "cleavable linker" and "protease" in the claims. The search identified 23 patents (Exhibit A). The first six patents listed are the topic of Exhibits B to G which provide the face sheet and patent claims of the respective patents.

U.S. Patent No. 7,511,124 (*see*, Exhibit B) was granted with a number of claims (e.g., claims 10, 43, 47,48, and 49 which set forth generically a "biologically releasable bond" and/or "selectively cleavable linker." Support for such in the '124 specification is principally found in a paragraph which recites:

> If desired, the Tissue Factor dimers or multimers may be joined via a biologically-releasable bond, such as a selectively-cleavable linker or amino acid sequence. For example, peptide linkers that include a cleavage site for an enzyme preferentially located or active within a tumor environment are contemplated. Exemplary forms of such peptide linkers are those that are cleaved by urokinase, plasmin, thrombin, Factor IXa, Factor Xa, or a metalloproteinase, such as collagenase, gelatinase or stromelysin.

The next listed patent is U.S. Patent No. 7,501,484. This patent which concerned constructs targeting neuronal cells was granted with dependent claim which set forth a chimeric polypeptide having a peptide linker cleavable by a generic neuronal protease (*see*, Exhibit C).

The next listed patent, U.S. Patent No. '833, is related to the '124 patent and also issued with claims drawn in part to a "biologically releasable bond" and/or "selectively cleavable linker" (*see*, Exhibit D).

The next listed patent, the '865 patent, although presenting a claim broadly drawn in part to an enzymatically cleavable linker is not much on point. (Exhibit E)

The next listed patent, U.S. Patent No. '909, is related to the '124 and '833 patents and also issued with claims drawn in part to a "biologically releasable bond" and/or "selectively cleavable linker" (*see*, Exhibit F).

The next listed patent U.S. Patent No. 7,279,564 also enjoys claims setting forth generic protease cleavable peptide linker subject matter (*see*, claims 10 to 12 of Exhibit G). Support for this subject mater is found in the '564 specification which recites

> As used herein, "third DNA sequences" comprise DNA sequences encoding a cleavable linker polypeptide. Such sequences include those which encode the prosequence of glucoamylase, the prosequence of bovine chymosin, the prosequence of subtilisin, prosequences of retroviral proteases including human immunodeficiency virus protease and DNA sequences encoding amino acid sequences recognized and cleaved by trypsin, factor X.sub.a collagenase, clostripin, subtilisin, chymosin, yeast KEX2 protease, Aspergillus KEXB and the like. See e.g. Marston, F. A. O. (1986) Biol. Chem J. 240, 1-12. Such third DNA sequences may also encode the amino acid methionine that may be selectively cleaved by cyanogen bromide. It should be understood that the third DNA sequence need only encode that amino acid sequence which is necessary to be recognized by a particular enzyme or chemical agent to bring about cleavage of the fusion polypeptide. Thus, the entire prosequence of, for example, glucoamylase, chymosin or subtilisin need not be used. Rather, only that portion of the prosequence which is necessary for recognition and cleavage by the appropriate enzyme is required.

Older patents with such protease cleavable linkers are listed. The last three patents listed in Exhibit A are the topic of Exhibits H to J which provide the face sheet and patent claims of the respective patents. The third from the last listed patent, U.S. Patent No. 5,679,543, has claims drawn in part to generic protease cleavable linkers (*see*, Exhibit H) claims 1, 17 and 40). The second-to-last of the 23 patents listed is U.S. Patent No. 5,654,176 which was granted in 1997. The '176 patent granted with claims (*see*, claims 3 to 5 of Exhibit I) drawn in part to fusion proteins comprising a generic protease cleavable peptide linker.

The earliest patent listed is the U.S. Patent No. 4,671,958 patent (see, Exhibit J) which was granted with claims (*see*, claims 24 and 29) drawn in part to chimeric molecules comprising an antibody and a drug connected by linkers (peptide and non-peptide) which were cleavable at the point of attachment to the drug by activated serum complement or serum proteases.

Accordingly, the Applicants submit that the protease recognition site subject matter of the instant claims is enabled and well within the ability of persons of ordinary skill in the art to practice without undue experimentation.

Cyclization and oxidation of amino acids of the donor and acceptor protein moieties to produce the "coupling of the donor and acceptor protein moieties

In response to the Examiner's allegation that "the specification does not provide guidance as to covalent binding occurring via cyclization and oxidation of amino acids of the donor and acceptor protein moieties, or via any other methods considered to produce the "coupling of the donor and acceptor protein moieties", the Applicants respectfully point out that the currently pending claims include the proviso that "*the donor moiety, the linker polypeptide, and the acceptor moiety are fused in a single amino acid sequence.*" Thus, the only embodiments embraced by the currently pending claims, are tandem proteins linked through amino acids contained in the same polypeptide, *i.e.* translated from a single polynucleotide or synthesized in a contiguous fashion. As such, the GFP donor and acceptor moieities are *not* coupled by the cyclization and oxidation of their fluorophore residues. Rather, it is the donor and acceptor fluorophores which are formed by cyclizations and oxidations occurring within each of the donor acceptor GFP moities of the fusion protein.

Applicants respectfully point out that the specification frequently teaches acceptor and donor GFP proteins can be 'coupled' as fusion proteins. For example, in Figure 2, in lines 28 to 30 on page 2 of the specification as filed, in lines 15 to 17 on page 29 of the specification as filed, in the paragraph bridging pages 31 and 32 of the specification as filed, and in Examples 1 and 2 of the specification. As further illustrated, in many of the patents of Exhibit A, fusion protein technology is advanced and requires no undue experimentation to be practiced. Indeed,

a search of the PTO database for claims which recite the phrase "fusion protein" identified 2822 patents (Exhibit G), the earliest of which was granted in *1982*.

With respect to the allegedly absent guidance with respect to achieving the cyclization and oxidation of the amino acids of each GFP moiety, the specification teaches at paragraph 87: "Recombinant fluorescent protein can be produced by expression of nucleic acid encoding the protein in E. coli. The fluorophore of Aequorea-related fluorescent proteins results from cyclization and oxidation of residues 65-67." An Applicant need not teach what is known in the art. Heim et al. (already of record, see Abstract, and incorporated by reference by virtue of paragraphs 49 and 122 in view of paragraph 140 of the specification as published) teaches that the cyclization occurs in the presence of oxygen and is either spontaneous or requires cellular factors which appear to be ubiquitous. Thus no additional details were required to practice this aspect of the invention. Indeed, Examples 1 and 2 show the method working without the performance of any additional steps particularly being required to achieve the cyclization and oxidation of donor and acceptor GFP residues 65-67 to obtain the donor and acceptor fluorophores.

Alleged lack of guidance as to what portion of the sequence is to be conserved and what the embraced mutations are.

The Office Action relies upon Heim et al. as showing that some GFP positions are critical to the practice of the invention. The Applicants note that Heim et al. was incorporated by reference into the instant specification (*see*, the paragraph bridging pages 14 and 15 by virtue of the recital bridging pages 45 and 46). As such, the ordinary practitioner in the art would have benefits of all its teachings, including those as to the critical portion of the molecule.

Furthermore, the Heim et al. reference does not support the Examiner's use of it. While the Examiner notes that some mutations resulted in weakly fluorescent proteins, the question of enablement does not turn on how well a construct works but, rather, whether it works at all. Moreover, Heim et al. shows just how little experimentation is required to make and identify a large number of mutant GFP proteins which would be suitable for use according to the invention.

The Applicants further call the Examiner to U.S. Patent Application Serial No. 08/706,408 which was incorporated by reference (*see*, the paragraph bridging pages 14 and 15 by virtue of the recital bridging pages 45 and 46). This application discloses up to **47** specific mutations which can be made in GFPs ands sets forth GFP X—ray crystallography data showing the structure of the protein at an atomic level (see, Figs. 1 to 5) and 3-dimensional structural models clearly showing those residues nearest the fluorophore. The teaching of the high-resolution crystal structure of GFP in the instant specification, as incorporated by reference, facilitates the selection of additional mutations that would not significantly alter the fluorescent properties in a variant of an *Av*GFP-rp. The provided structure and description of its functional domains serves to identify regions which may be individually modified to achieve distinct purposes without an expectation of affecting the functioning of other domains. It also identifies individual residues and their environments which further provides a person of ordinary skill in the art with opportunities to conservatively substitute residues with little expectation of greatly altering the activity of a GFP. Applicants respectfully submit, that any structural biologist of average skill in the art, would readily be able to generate a plethora of silent mutations in a variant of an *Av*GFP-rp, through manual inspection of the crystal structure alone. Three classes of residues where conservative mutations would result in little to no consequence are those distal to the chromophore, those whose side chains are solvent exposed and do not contribute directly to the electron environment of the chromophore, and those found in loop regions connecting the beta-strands in the ternary structure. As evidenced by the crystal structure found in Figure 1 of the '408 specification, these three classes of residues comprise well over 25% of the GFP protein. In addition, the '408 specification teaches how to use the crystallography data to design mutants at pp. 20 to 22 where substitutions can result in altered fluorescent characteristics.

Additionally, PCT/US95/14692, which was also incorporated by reference (*see*, with respect to the incorporation, the paragraphs bridging pages 14 and 15 and 45 and 46, respectively, of the Applicant's specification) discloses additional GFP mutant proteins of from 2 to 7 substitutions which have altered fluorescent properties (see, p. 19, table 2 of the PCT/US95/14692 specification).

The Applicants thank the Examiner for acknowledging that the 95% sequence identity subject matter is enabled. However, the enablement of the 85% sequence identity subject matter remains at issue. The Office Action cites Guo et al. for the proposition that "*the art sets forth that a third of single amino acid changes would completely inactivate the average protein and the more substitutions made the more probability that the protein will be inactivated. Thus, this gives the sense of what one of skill in the art can expect when a claim embraces fragments with up to 10, 20, 30, 40 or more amino acid changes and how many mutants one of skill in the art can test in such an endeavor (see Guo et al., PNAS, vol. 101, no.25, pages 9205-9210,2004).*"

However, the Applicants note that GFP is *not* the average protein posited by the Office Action, nor is it an enzyme as studied by Guo et al. Lawrence has shown that GFP is extremely tolerant of substitutions. The ability of persons of ordinary skill in the art to extensively re-engineer the GFP has been demonstrated in fact (see, Lawrence et al., J. Am. Chem. Soc. 129:10110-10112 (2007). Starting from a modified GFP which has about 7 substitutions already from the wild-type GFP, Lawrence et al. re-engineered the protein to obtain several GFP's having up to an additional 36 amino acids ***non-conservatively*** substituted (i.e., negatively charged or neutral amino acids were replaced by an Arg or Lys). In another of the modified GFP proteins, 15 negatively charged or neutral amino acids were replaced by a Glu or Asp. Over all, modified GFPs differing in overall charge from +48 to -30 were made and found to have a GFP fluorescence activity! These results clearly show that the GFP is *extremely* tolerant to substitutions and that highly diverse variants can be readily obtained by persons of ordinary skill in the art. The Lawrence work clearly shows that any contrary expectation based upon the Guo et al. art simply does not apply to GFP. Accordingly, the Office Action's reliance upon Guo is misplaced.

In view of the extensive disclosure of compatible mutations in the specification, the crystallographic and functional analyses provided therein, the advanced state of the art with respect to the manipulations needed to practice the claimed invention, the relative simplicity with which mutants may be screened in truly enormous numbers as evidenced by Heim et al., and the

demonstrated ability of others using the very protein to obtain useful fluorescent proteins with even less than 85% sequence identities, the Applicants submit that the claimed invention can be practiced with an amount of experimentation which is *not* undue but, rather, *routine* in the art.

Accordingly, in view of the ample evidence now provided, the Applicants respectfully request that the above grounds for rejection be reconsidered and withdrawn.

Response to the rejection of claims 79-84 and 91-99 under the judicially created doctrine of obviousness-type double patenting over claims 1-13 and 43-44 of U.S. Patent No. 6,803,188

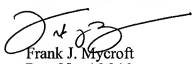
Without acquiescing on the merits, in the event that claims are allowed in this application and a terminal disclaimer must be provided, Applicants would submit a Terminal Disclaimer in accordance with 37 C.F.R. 1.132(c). Applicants therefore request that the rejections of be held in abeyance until such time as a claim is otherwise deemed to be in condition for an allowance.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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